PATENTS
Atty. Docket No. 47508-423 (HYZ-423)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Zhou *et al.*	Art Unit:	1635
Serial No.:	09/283,431	Examiner:	K.A. Lacourciere
Filing Date:	April 1, 1999		
Title:	**Mixed Backbone Oligonucleotides Containing POPS Blocks to Obtain Reduced Phosphorothioate Content**		

Box AF
Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF DR. EKAMBAR R. KANDIMALLA UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Ekambar R. Kandimalla, declare as follows:

1. I am currently the Senior Director of Research at Hybridon, Inc., located at 345 Vassar Street in Cambridge, MA (02139).

2. I obtained my B.S. in Chemistry in 1978, my M.S. in Biochemistry in 1980, and my Ph.D. in Chemistry in 1984, all three degrees from Andhra University in India. I have been involved in oligonucleotide research, including antisense oligonucleotide research, since 1992. I have authored or co-authored more than 100 full-length journal articles, book chapters, and reviews, and have given oral presentations at more than 40 conferences. I am an inventor on ten issued U.S. patents in the field of oligonucleotides, which include patents on chemically modified oligonucleotide compositions in general and modified antisense oligonucleotides in particular. My *curriculum vitae*, along with a list of these publications and presentations, is enclosed herewith as Appendix A.

3. I am familiar with the above-referenced patent application, the presently amended claims presented herewith, the Final Office Action dated March 2, 2005, and the references Metelev *et al.* (U.S. 6,143,881) and Ghosh *et al.* ((1993) Anti-Cancer Drug Design 8(1): 15-32).

4. I understand that the currently presented claims 4-25 are drawn to hybrid and inverted hybrid oligonucleotides having particular arrangements of regions of POPS block sequence and regions of 2'-O-modified ribonucleosides.

5. I further understand that the Final Office Action dated March 2, 2005, includes a rejection of these claims in view of the combined teachings of Metelev *et al.* (U.S. 6,143,881) and Ghosh *et al.* ((1993) Anti-Cancer Drug Design 8(1): 15-32). In particular, this Final Office Action states that "it would have been obvious to one of ordinary skill in the art to make a hybrid oligonucleotide comprising a region of alternating phosphorothioate and phosphodiester linkages, as taught by Ghosh *et al.*, with a region of 2'-O-substituted ribonucleotides, as taught by Metelev *et al.*...(and that)...Metelev *et al.* provide a motivation to do so, teaching that hybrid oligonucleotides comprising phosphorothioate and phosphodiester linkages and 2'-O-subtituted ribonucleotides and deoxyribonucleotide regions have superior properties of duplex formation, RNase H activation and nuclease resistance when used as an antisense molecule." I disagree with these statements for the following reasons.

6. I was a person of skill in the art at the time of the instant invention. Furthermore, I was aware actually aware of both the Metelev *et al.* and the Ghosh *et al.* references, and yet I would not have been motivated to combine their teachings in the suggested manner. In particular, Metelev *et al.* teaches (Table 1, at column 11, lines 20-24) that 2'-O-Me modifications solve the problem of reduced hybridizing ability seen with all-PS oligonucleotides by enhancing their duplex stability. Similarly, Ghosh *et al.* teaches that duplex stability of all-PS oligonucleotides is reduced relative to all-PO oligonucleotides, but that the use of PS-PO copolymers provides for intermediate hybridizing abilities. Hence, there was no motivation for the skilled artisan to combine the mixed PS-PO copolymers of Ghosh *et al.* with the 2'-OMe modifications of Metlev *et al.*, because Metlev *et al.* teaches that the problem of reduced duplex

stability in all-PS oligonucleotides is already solved by the introduction of 2'-OMe modifications. Furthermore, there was good reason not to combine the PS-PO copolymers of Ghosh *et al.* with the all-PS, 2'-OMe-modified oligonucleotides of Metelev *et al.* because Ghosh *et al.* teaches that the introduction of PO linkages increases nuclease susceptibility. Therefore, it would there was no motivation to combine the teachings of Metelev *et al.* with the teachings of Agrawal et al. to arrive at the invention described by the currently-presented claims.

7. I further note that, not only was there no motivation to combine the cited references, but there was also no reasonable expectation of success in using such a combination. Indeed, there was knowledge in this field at the time of the invention that would have made researchers expect particular problems in using the claimed oligonucleotides.

8. I have further reviewed the experimental data previously submitted in Applicants' Amendment dated February 24, 2004 and believe it demonstrates unexpected, advantageous properties of the claimed improved hybrid oligonucleotides. In particular, this experimental analysis shows that the hybrid oligonucleotides of the invention have unexpectedly desirable properties of nuclease stability, while uniquely avoiding deleterious immune-mediated toxicity (see Exhibits A-1 through A-6).

Table 1, shown below, summarizes the results of these studies. Briefly, the results show that an unmodified, all-phosphodiester-linked oligonucleotide (GEM 231), while able to avoid undesirable activation of complement, is not at all stable against nucleases found in bovine serum (compare Exhibit A-1 to A-2 (0% stable)). In contrast, GEM 231 oligonucleotide containing both 2'-O-Me-modified nucleosides and an all-phosphorothioate backbone is very resistant to nuclease degradation (compare Exhibit A-3 to A-4 (95% stable)).

However this all-phosphorothioate oligonucleotide is immunogenic and would cause deleterious activation of complement in a treated subject. Surprisingly, the introduction of alternating phosphodiester/ phosphorothioate linkages into GEM 231 allows the structure to

retain most of its nuclease stability (compare Exhibit A-5 to A-6 (57% stable)), while avoiding the deleterious immune effects seen with all-phosphorothioate oligonucleotides, as well as the decreased duplex stability seen with all-phosphorothioate oligodeoxyribonucleotides.

TABLE 1

EXHIBIT	OLIGONUCLEOTIDE	STRUCTURE[1]	RELATIVE STABILITY[2]
A-1 & A-2	GEM 231-PO	5'-GoCoGoUoGoCoCoToCoCoToCoAoCoUoGoGoC-3'	0%
A-3 & A-4	GEM 231 -2'-O-Me-all-PS	5'-GsCsGsUsGsCsCsTsCsCsTsCsAsCsUsGsGsC-3'	95%
A-5 & A-6	GEM 231 -2'-O-Me-POPS	5'-GsCoGsUoGsCoCsToCsCoTsCoAsCoUsGoGsC-3'	57%

1. Internucleoside linkages are indicated as "s" for phosphorothioate linkages and "o" for phosphorothioate linkages; 2'-O-methylribonucleoside residues are underlined.

2. Relative stability is the percent of intact oligonucleotide remaining following incubation with 10% fetal bovine serum at 37° C for 24 hours.

Therefore, it is my belief that the above-presented data demonstrates unexpected and advantageous properties of the claimed improved hybrid oligonucleotides.

9. In conclusion, it is my belief that, at the time of this Application was filed, one of ordinary skill in the field of oligonucleotides in general, and antisense oligonucleotides in particular, would not have been motivated to combine the teachings of Metelev *et al.* and Ghosh *et al.* to arrive at the invention claimed herewith. Furthermore, a person of skill in the art at the time of the invention would not have had a reasonable expectation of success in actually using this combination. In addition, it is my belief that the above-described experiments evidence the unexpected and advantageous properties of the claimed improved hybrid oligonucleotides.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date AUG 30, 2005

Ekambar R. Kandimalla, Ph.D.

Appendix A

EKAMBAR R. KANDIMALLA
Hybridon, Inc.
345 Vassar Street
Cambridge, MA 02139.

Experience

07/2003 — Present	Senior Director of Research, Hybridon, Inc. Coordinating internal and external research programs using antisense and IMO technologies in disease models of interest. Coordinating patent filings. Coordinating technical aspects of IMO manufacturing.
08/1999 — 06/2003	Director of Antisense and Functional Genomics, Hybridon, Inc. Application of antisense technology for functional genomics - Antisense oligonucleotide design and target validation, fluorescence based PCR probes and primers, CpG oligonucleotide-based immunotherapeutics.
07/1993 - 07/1999	Senior Research Scientist, Hybridon, Inc. Design, synthesis, biophysical and biochemical studies of antisense and triplex-forming oligonucleotides; Studies of the interaction of oligos with biological macromolecules; Solid phase attachment of oligos for diagnostic and analytical uses.
06/1992 - 06/1993	Research Scientist, Hybridon, Inc. Design, synthesis, biophysical and biochemical studies of antisense oligonucleotides
09/1987-06/1992	Research Associate, Department of Chemistry, University of Alberta. Molecular recognition of nucleic acids; Design, and synthesis of sequence-specific minor groove binding peptide antibiotics as anticancer and gene expression control agents; Biophysical, biochemical and molecular biological studies on DNA-binding agents and proteins.
01/1985-09/1987	Research Associate, Molecular Biophysics Unit, Indian Institute of Science. Design, synthesis and nucleic acid binding studies of new analogs of DNA binding peptide antibiotics netropsin and distamycin.
02/1981-12/1984	Jr. and Sr. Research Fellow, School of Chemistry, Andhra University. Development of new reactions and reagents for phenols, aminophenols, and amino acids.

Expertise

Chemistry and biology of nucleic acids and oligonucleotides - drug design and discovery, molecular recognition of nucleic acids, drug/protein-nucleic acid interactions, nucleic acid and oligonucleotide chemistry and synthesis, nucleic acid therapeutics, antisense and CpG DNA, *in vitro* and *in vivo* disease models.

Education

Ph.D.	Chemistry, Andhra University, India	1984
M.Sc.	Biochemistry, Andhra University, India	1980
B.Sc.	Chemistry (Major) & Botony and Zoology, Andhra University, India	1978

Awards/Fellowships

1986-1987	Research Associate	Council of Scientific and Industrial Research, India.
1981-1984	Jr. & Sr. Research Fellowship	Council of Scientific and Industrial Research, India.
1978-1980	National Merit Scholarship	Government of India.

Publications, presentations, and patents

Publications	Over 100 in peer-reviewed journals, including review articles and book chapters.
Conferences	Over 40 persentations.
Patents	10 issued.

List of selected publications

86. *DC.McManus, CA.Lefebvre, GC.Horvat, M. St.-Jean,* ***ER.Kandimalla,*** *S.Agrawal, S.Morris, JP.Durkin & EC.LaCasse.* Loss of XIAP protein expression by RNAi and antisense approaches sensitizes cancer cells to chemotherapy-induced apoptosis. Oncogene (in press, 2004).

85. *JL.Bjersing, A Tarkowski,* ***ER Kandimalla,*** *S Agrawal & LV.Collins.* Impact of site-specific nucleobase deletions on the inflammatogenicity of DNA. Inflammation (in press, 2004).

84. *FG.Zhu,* ***ER.Kandimalla,*** *D.Yu, JX.Tang.* Modulation of ovalbumin induced Th2 responses by second generation immunomodulatory oligonucleotides in mice. *Int. Immunopharmacol.* **4**, 851-862, 2004.

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82. *W.Jiang, CF.ReicIII, D.Yu,* ***ER.Kandimalla,*** *S.Agrawal & DS.Pisetsky.* Induction of immune activation by a novel immunomodulatory oligonucleotide without thymocyte apoptosis. *Biochem. Biophys. Res. Commun.* **318**, 60-66, 2004.

81. ***ER.Kandimalla,*** *RK.Pandey & S.Agrawal.* Hybridization-based fluorescence assay allows quantitation of single-stranded oligodeoxynucleotides in low nanomolar range. *Anal. Biochem.* **328**, 93-95, 2004.

80. *DK.Agrawal, J.Edwan,* ***ER.Kandimalla,*** *D.Yu, L.Bhagat, D.Wang & S.Agrawal.* Novel Immunomodulatory oligonucleotides (IMOs) prevent development of allergic airway inflammation and airway hyperresponsiveness in Asthma. *Int. Immunopharmacol.* **4**, 127-138, 2004.

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77. ***ER.Kandimalla*** *& S.Agrawal.* Chemistry of CpG DNA. In *Curr. Prot. Nucleic Acids Chem.* (Ed. S. Beucauge), pp 4.13.1-4.13.13, John-Wiley, New York, 2003.

76. ***ER.Kandimalla***, L.Bhagat, FG.Zhu, D.Yu, YP.Cong, D.Wang, JX.Tang, JY.Tang, CF.Knetter, E.Lien & S.Agrawal. A dinucleotide motif in oligonucleotides shows potent immunomodulatory activity and overrides species specific recognition observed with CpG motif. *Proc. Natl. Acad. Sci. USA.* **100**, 14303-14308, 2003.

75. YP.Cong, SS.Song, L.Bhagat, RK.Pandey, D.Yu, ***ER.Kandimalla*** & S.Agrawal. Self-stabilized CpG DNAs optimally activate human B cells and plasmacytoid dendritic cells. *Biochem. Biophys. Res. Commun.* **310**, 1133-1139, 2003.

74. ***ER.Kandimalla***, L.Bhagat, YP.Cong, RK.Pandey, D.Yu, Q.Zhao & S.Agrawal. Secondary structures in CpG oligonucleotides affect immunostimulatory activity. *Biochem. Biophys. Res. Commun.* **306**, 948-953, 2003.

73. ***ER.Kandimalla***, L.Bhagat, D.Wang, D.Yu, FG.Zhu, J.Tang, H.Wang, P.Huang, R.Zhang & S.Agrawal. Divergent synthetic nucleotide motif recognition pattern: design and development of potent immunomodulatory oligodeoxyribonucleotide agents with distinct cytokine induction profiles. *Nucleic Acids Res.*, **31**, 2393-2400, 2003.

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68. D.Yu, F-G.Zhu, L.Bhagat, H.Wang, ***ER.Kandimalla***, R.Zhang & S.Agrawal. Potent CpG oligonucleotides containing phosphodiester linkages: *In vitro* and *in vivo* immunostimulatory properties. *Biochem. Biophys. Res. Commun.* **297**, 83-90, 2002.

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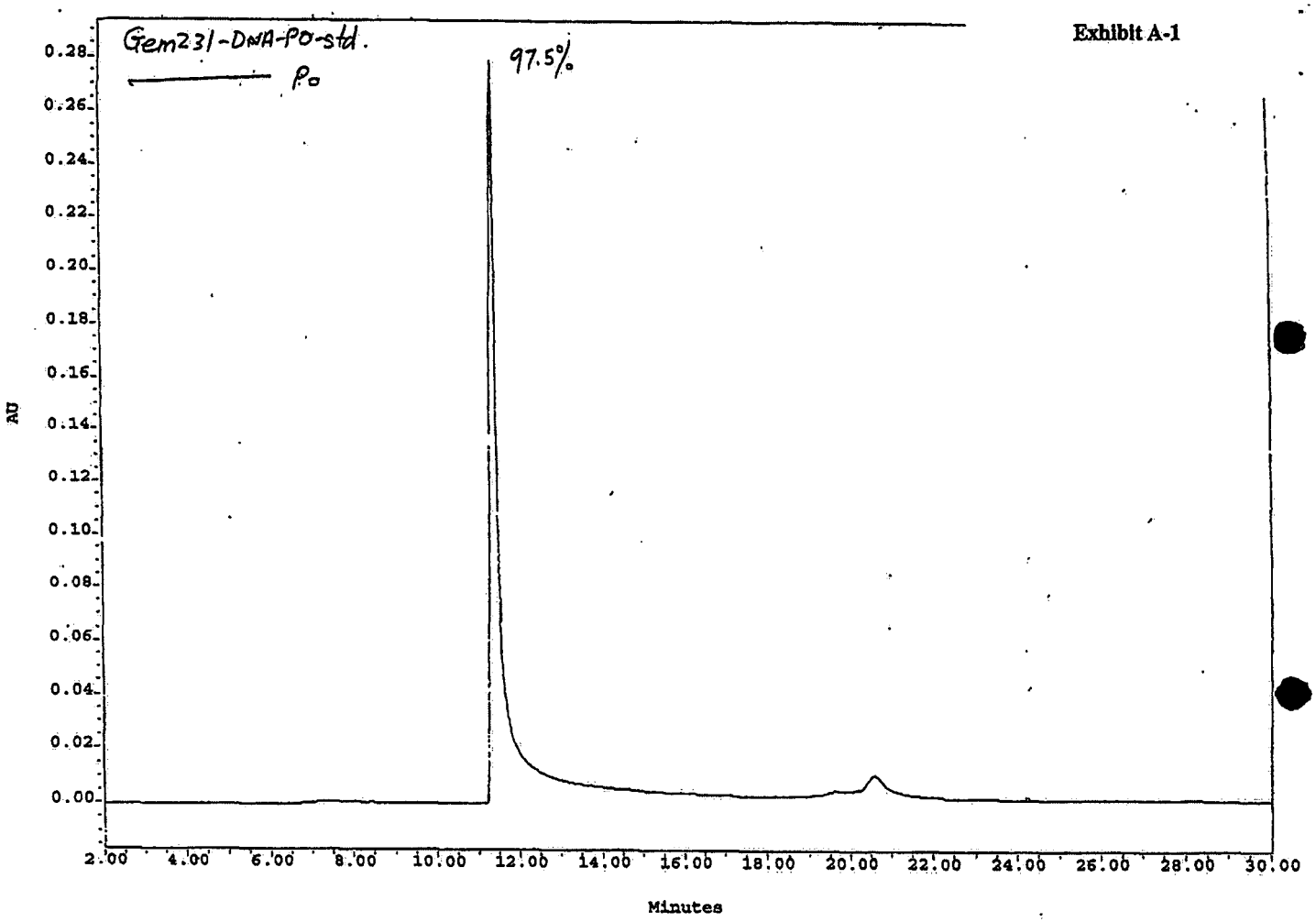
Exhibit A-1



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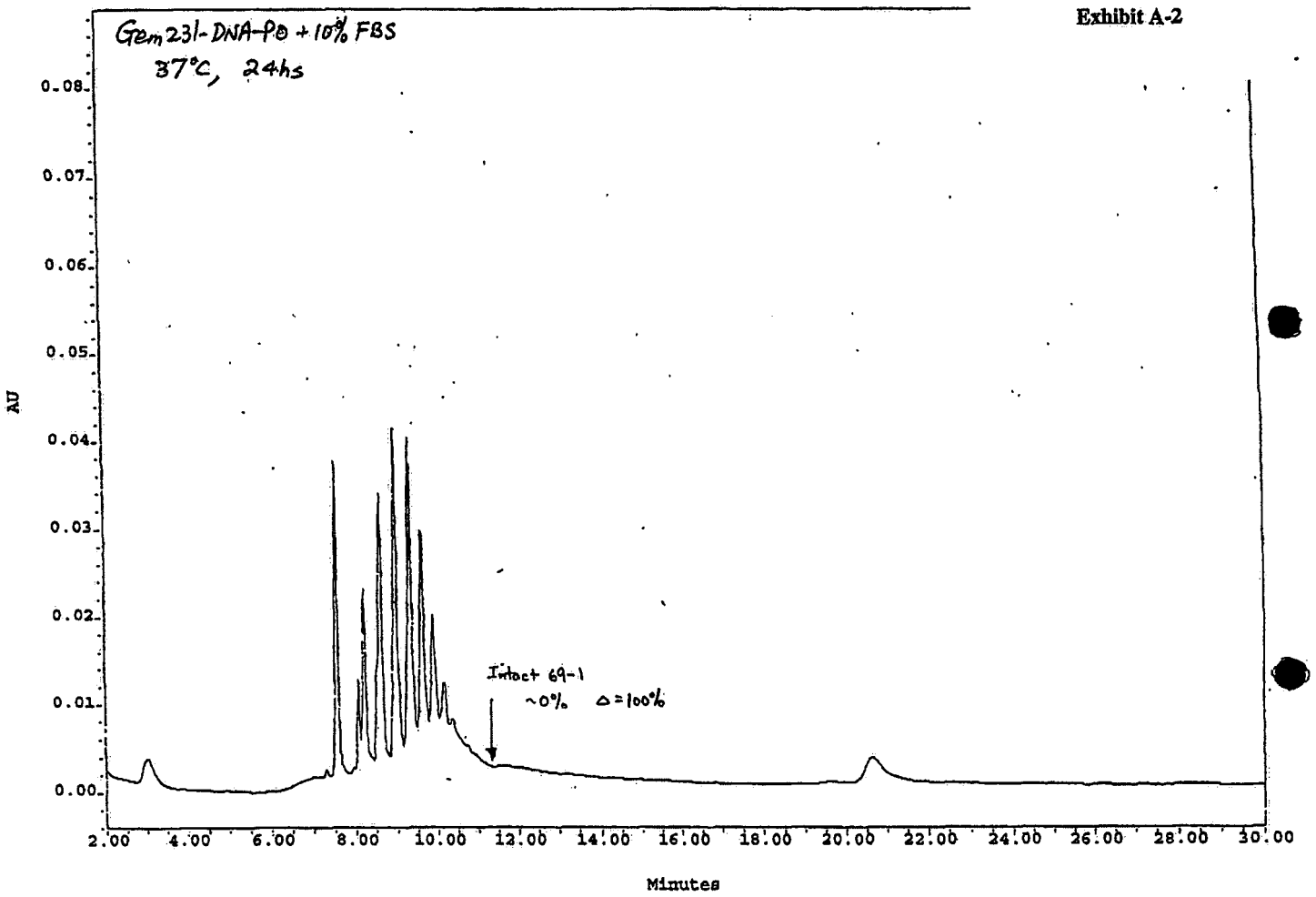

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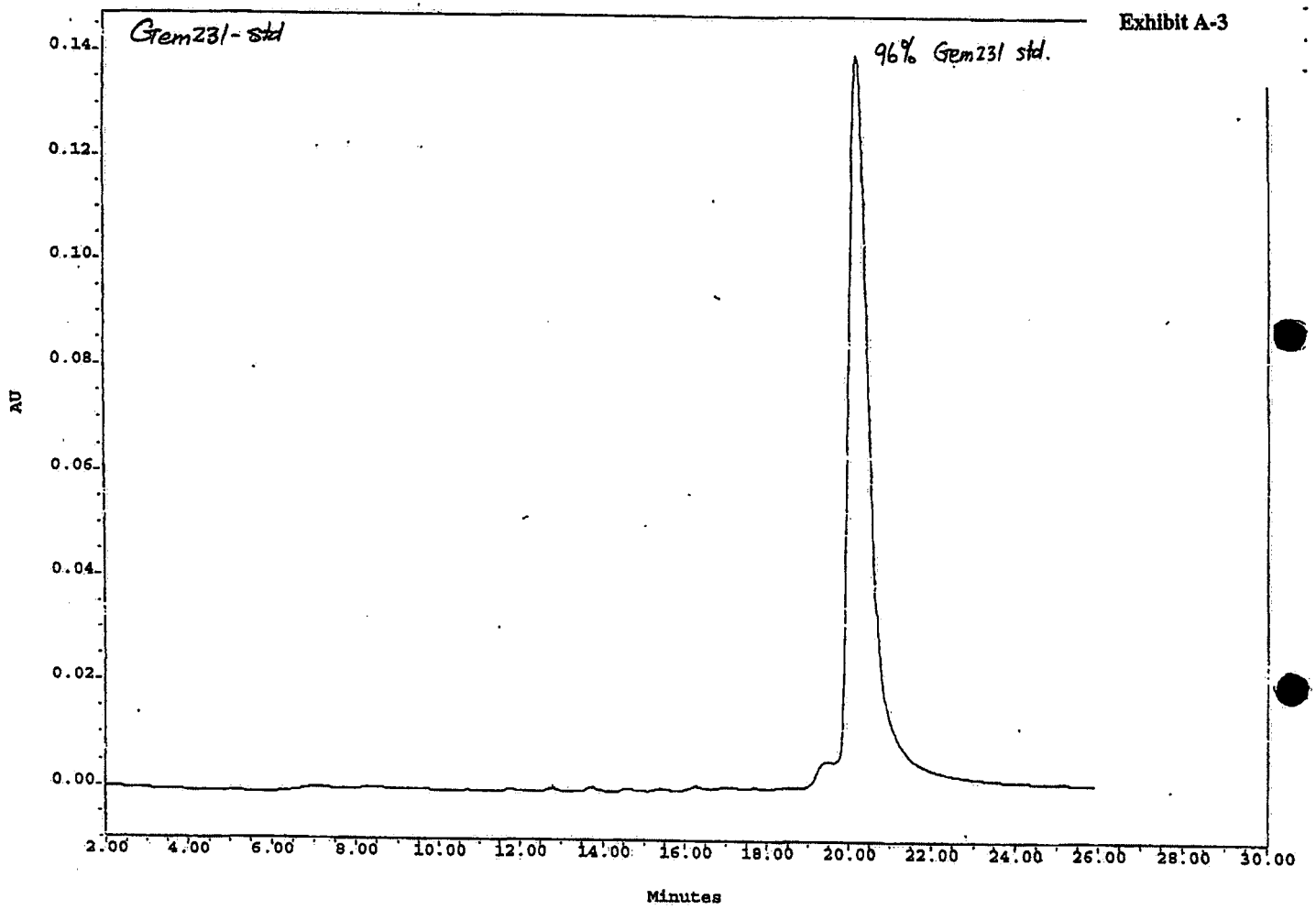

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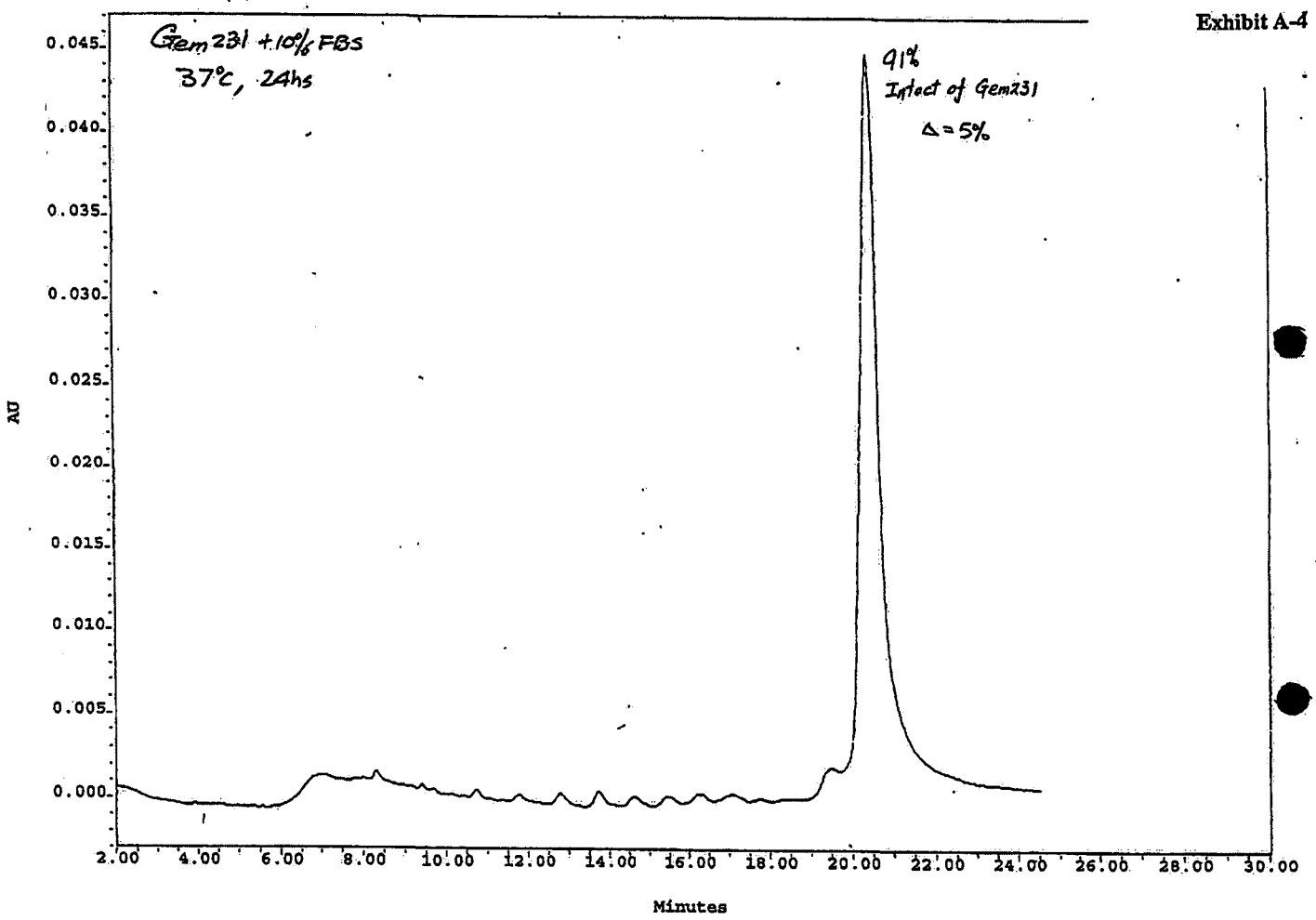

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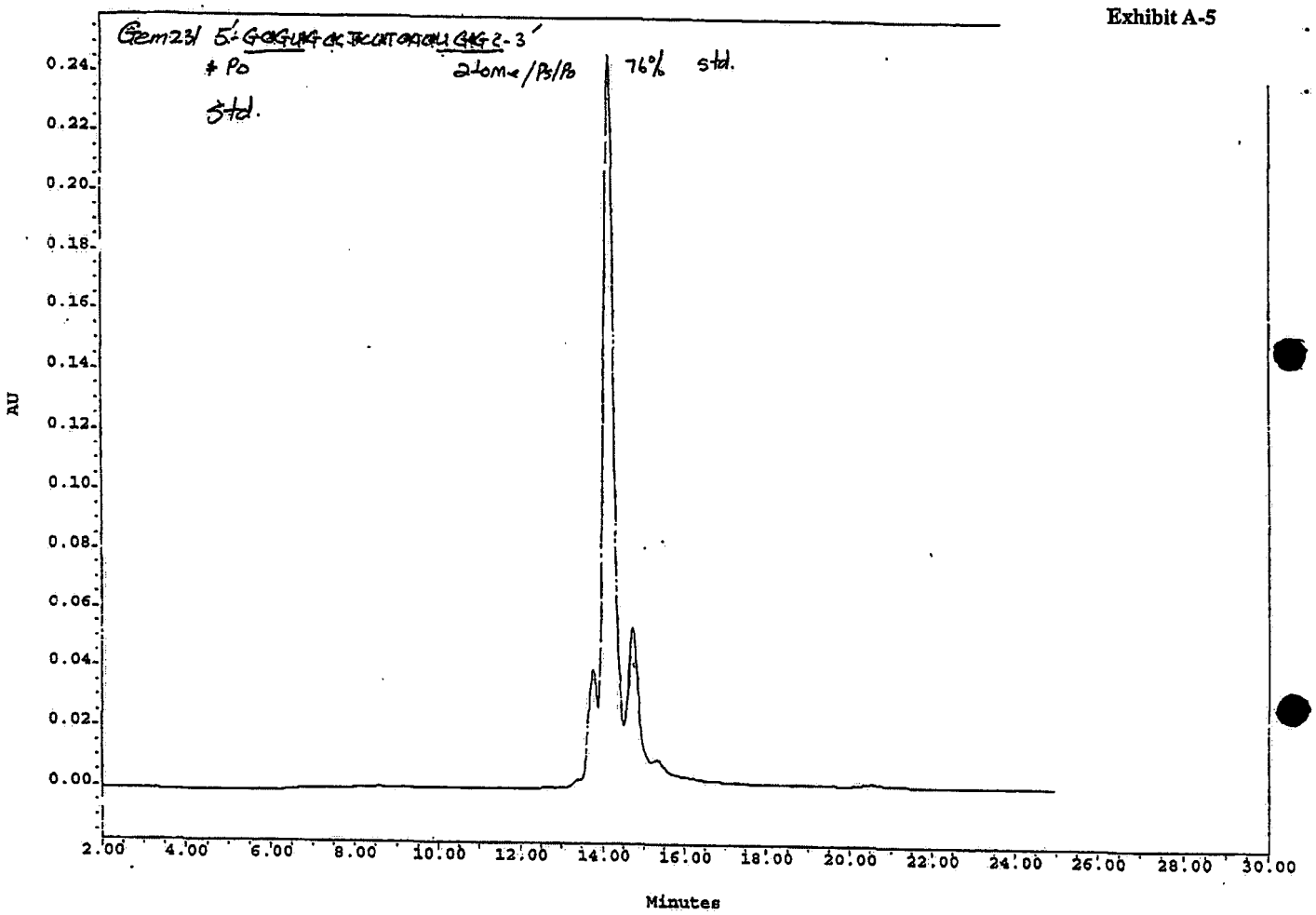

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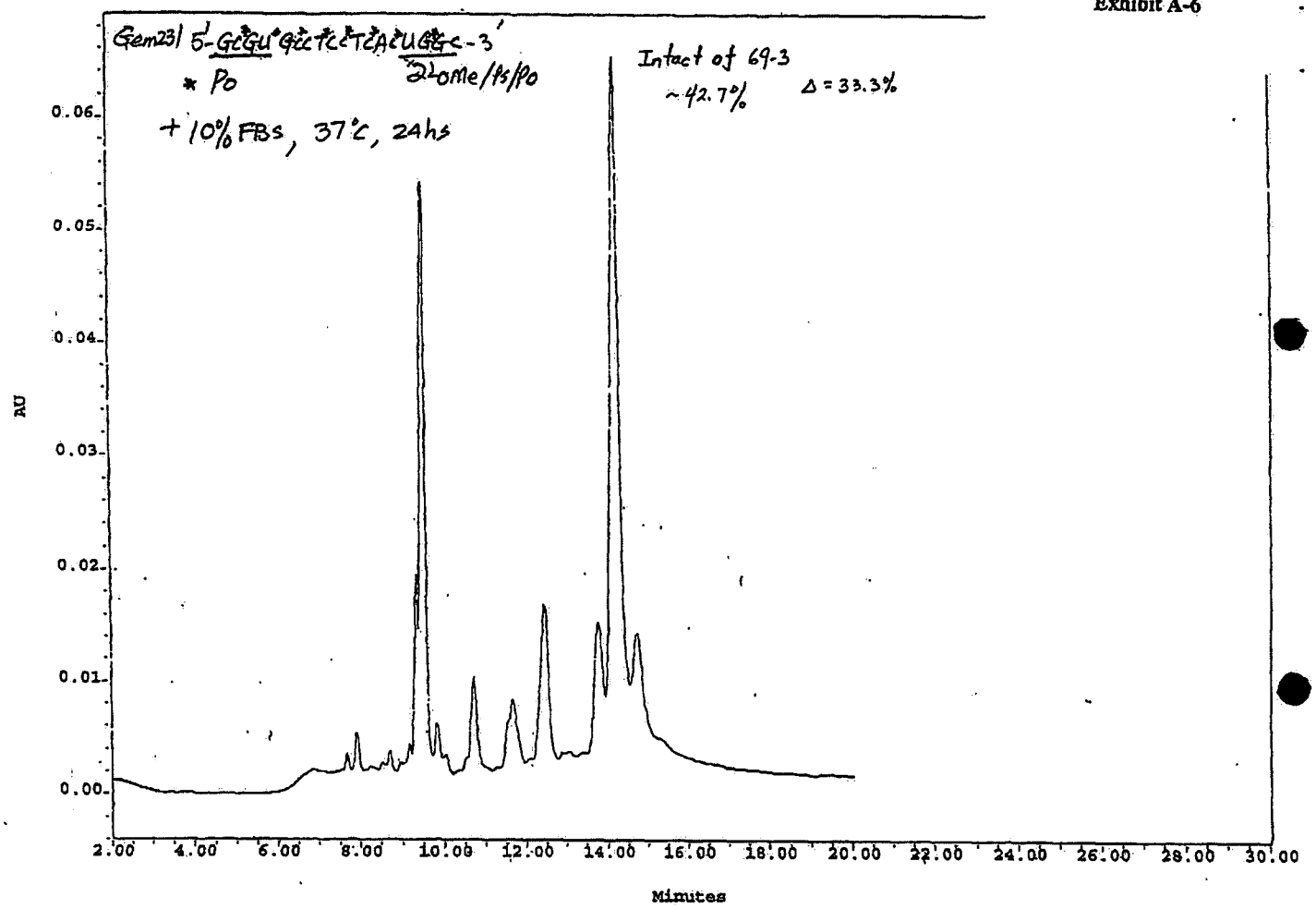

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